

## CLAIMS

We claim:

5      1. A set of features comprising oligophosphodiester probes, wherein said features comprise

(a) hybridization features comprising hybridization probes that selectively hybridize to a detectably labeled target nucleotide sequence, and

10      (b) background features comprising background probes that do not selectively hybridize to said target nucleotide sequence.

2. The set of claim 1, wherein said hybridization probes and said background probes are bound to a surface or are present in solution.

15      3. The set of claim 2 wherein said surface is an array surface.

4. The set of claim 1, wherein the background probes comprise empirically observed inactive probes, probes forming stable intramolecular structures, short probes, reverse polarity nucleotide analogs, abasic phosphodiester or modified nucleotidic units.

20      5. The set of claim 4, wherein the background probes comprise empirically observed inactive probes selected from the group consisting of

CAGAGGAAGAGAATCTCCGCAAGAA (SEQ ID NO: 5);

GAATCTCCGCAAGAAAGGGGAGCCT (SEQ ID NO: 6);

25      CGAGCTGCCCCCAGGGAGCACTAAG (SEQ ID NO: 7);

CCAGGGAGCACTAAGCGAGCACTGC (SEQ ID NO: 8);

TGAATGAGGCCTTGGAACTCAAGGA (SEQ ID NO: 9);

AAGGATGCCCAGGCTGGGAAGGAGC (SEQ ID NO: 10);

AGGCTGGGAAGGAGCCAGGGGGGAG (SEQ ID NO: 11);

30      GGAGCCAGGGGGGAGCAGGGGCTCAC (SEQ ID NO: 12);

TGGGCTACACTGAGCACCAGGTGGT (SEQ ID NO: 13);

AATATGATGACATCAAGAAGGTGGT (SEQ ID NO: 14);

ATCCCTGAGCTAGACGGGAAGCTCA (SEQ ID NO: 15);  
 AACTGTGGCGTGATGGCCGCGGGGC (SEQ ID NO: 16);  
 GTGTGAACCATGAGAAGTATGACAA (SEQ ID NO: 17); and  
 TTCGTCATGGGTGTGAACCATGAGA (SEQ ID NO: 18).

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6. The set of claim 4, wherein the background probes comprise probes forming stable intramolecular structures, wherein the probes are selected from the group consisting of

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GCTAGCGAAAGCTAGC (SEQ ID NO: 24);  
 GCGAGCGAAAGCGAGC (SEQ ID NO: 25);  
 GCAGGCGAAAGCAGGC (SEQ ID NO: 26);  
 GCAGGGGAAAGCAGGC (SEQ ID NO: 27); and  
 GCATACCGAAGCAGGC (SEQ ID NO: 28).

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7. The set of claim 4, wherein the background probes comprise short probes, wherein the probes are selected from the group consisting of

AACCATGAGAAGTATGACAA (SEQ ID NO: 29);  
 TGAGAAGTATGACAA (SEQ ID NO: 30);  
 AGTATGACAA (SEQ ID NO: 31); and  
 GACAA (SEQ ID NO: 32).

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8. The set of claim 4, wherein the background probes comprise reverse polarity nucleotide analogs.

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9. The set of claim 4, wherein the background probes comprise abasic phosphodiester or modified nucleotidic units.

10. A method of detecting the presence and/or amount of a target nucleotide sequence in an analyte, said method comprising:

- (a) providing an analyte suspected of containing the target nucleotide sequence;
- (b) contacting an aliquot of said analyte suspected of containing said target nucleotide sequence with a set of features comprising oligophosphodiester probes, wherein

said target nucleotide sequence is labeled with a detectable label capable of generating a measurable signal, and further wherein said features comprise:

- (i) hybridization features comprising hybridization probes that selectively hybridize to said labeled target nucleotide sequence, and
- (ii) background features comprising background probes that do not selectively hybridize to said labeled target nucleotide sequence;
- (c) detecting an observed signal, wherein the observed signal is an amount of signal generated from contacting the target nucleotide sequence with said features comprising oligophosphodiester probes;
- (d) detecting a background signal, wherein said background signal is an amount of signal generated from said background features;
- (e) subtracting the background signal from the observed signal to determine the presence and/or amount of said target nucleotide sequence in said analyte.

11. The method of claim 10 wherein said hybridization probes and said background probes are bound to an array surface.

12. The method of claim 10, wherein the wherein said target nucleotide sequence is directly labeled with said detectable label.

13. The method of claim 10, wherein the wherein said target nucleotide sequence is indirectly labeled with said detectable label.

14. The method of claim 10, wherein said signal is detected by colorimetric, fluorimetric, chemiluminescent or bioluminescent techniques.

15. The method of claim 10, wherein the background probes comprise empirically observed inactive probes, probes forming stable intramolecular structures, short probes, reverse polarity nucleotide analogs, abasic phosphodiester or modified nucleotidic units.

16. The method of claim 15, wherein the background probes comprise

empirically observed inactive probes selected from the group consisting of

CAGAGGAAGAGAATCTCCGCAAGAA (SEQ ID NO: 5);  
 GAATCTCCGCAAGAAAGGGGAGCCT (SEQ ID NO: 6);  
 CGAGCTGCCCCCAGGGAGCACTAAG (SEQ ID NO: 7);  
 CCAGGGAGCACTAAGCGAGCACTGC (SEQ ID NO: 8);  
 TGAATGAGGCCTTGGAACTCAAGGA (SEQ ID NO: 9);  
 AAGGATGCCCAGGCTGGGAAGGAGC (SEQ ID NO: 10);  
 AGGCTGGGAAGGAGCCAGGGGGGAG (SEQ ID NO: 11);  
 GGAGCCAGGGGGGAGCAGGGGCTCAC (SEQ ID NO: 12);  
 TGGGCTACACTGAGCACCAGGTGGT (SEQ ID NO: 13);  
 AATATGATGACATCAAGAAGGTGGT (SEQ ID NO: 14);  
 ATCCCTGAGCTAGACGGGAAGCTCA (SEQ ID NO: 15);  
 AACTGTGGCGTGATGGCCGCGGGGC (SEQ ID NO: 16);  
 GTGTGAACCATGAGAAAGTATGACAA (SEQ ID NO: 17); and  
 TTCGTCATGGGTGTGAACCATGAGA (SEQ ID NO: 18).

17. The method of claim 15, wherein the background probes comprise probes forming stable intramolecular structures, wherein the probes are selected from the group consisting of

GCTAGCGAAAGCTAGC (SEQ ID NO: 24);  
 GCGAGCGAAAGCGAGC (SEQ ID NO: 25);  
 GCAGGCGAAAGCAGGC (SEQ ID NO: 26);  
 GCAGGGGAAAGCAGGC (SEQ ID NO: 27); and  
 GCATACCGAAGCACGC (SEQ ID NO: 28).

18. The set of claim 15, wherein the background probes comprise short probes, wherein the probes are selected from the group consisting of

AACCATGAGAAGTATGACAA (SEQ ID NO: 29);  
 TGAGAAGTATGACAA (SEQ ID NO: 30);  
 AGTATGACAA (SEQ ID NO: 31); and  
 GACAA (SEQ ID NO: 32).

19. The method of claim 15, wherein the background probes comprise reverse polarity nucleotide analogs.

20. The method of claim 15, wherein the background probes comprise abasic phosphodiester or modified nucleotidic units.

21. A method for estimating background noise in a nucleic acid hybridization assay, said method comprising providing a set of features comprising oligophosphodiester probes, wherein said features comprise hybridization features comprising hybridization probes that selectively hybridize to a target nucleotide sequence, and background features comprising background probes that do not selectively hybridize to said target nucleotide sequence; and subtracting the background signal, wherein said background signal is an amount of signal generated from said background features, from the observed signal, wherein the observed signal is an amount of signal generated from contacting the target nucleotide sequence with said features comprising oligophosphodiester probes.

22. The method of claim 21 wherein said hybridization probes and said background probes are bound to an array surface.

23. The method of claim 21, wherein said signal is detected by colorimetric, fluorimetric, chemiluminescent or bioluminescent techniques.

24. The method of claim 21, wherein the background probes comprise empirically observed inactive probes, probes forming stable intramolecular structures, short probes, reverse polarity nucleotide analogs, abasic phosphodiester or modified nucleotidic units.

25. The method of claim 24, wherein the background probes comprise empirically observed inactive probes selected from the group consisting of  
CAGAGGAAGAGAATCTCCGCAAGAA (SEQ ID NO: 5);  
GAATCTCCGCAAGAAAGGGGAGCCT (SEQ ID NO: 6);

CGAGCTGCCCCCAGGGAGCACTAAG (SEQ ID NO: 7);  
 CCAGGGAGCACTAAGCGAGCACTGC (SEQ ID NO: 8);  
 TGAATGAGGCCTTGGAAGTCAAGGA (SEQ ID NO: 9);  
 AAGGATGCCCAGGCTGGGAAGGAGC (SEQ ID NO: 10);  
 AGGCTGGGAAGGAGCCAGGGGGGAG (SEQ ID NO: 11);  
 GGAGCCAGGGGGGAGCAGGGGCTCAC (SEQ ID NO: 12);  
 TGGGCTACACTGAGCACCAGGTGGT (SEQ ID NO: 13);  
 AATATGATGACATCAAGAAGGTGGT (SEQ ID NO: 14);  
 ATCCCTGAGCTAGACGGGAAGCTCA (SEQ ID NO: 15);  
 AACTGTGGCGTGATGGCCGCGGGGC (SEQ ID NO: 16);  
 GTGTGAACCATGAGAAGTATGACAA (SEQ ID NO: 17); and  
 TTCGTCATGGGTGTGAACCATGAGA (SEQ ID NO: 18).

26. The method of claim 24, wherein the background probes comprise probes forming stable intramolecular structures, wherein the probes are selected from the group consisting of

GCTAGCGAAAGCTAGC (SEQ ID NO: 24);  
 GCGAGCGAAAGCGAGC (SEQ ID NO: 25);  
 GCAGGCGAAAGCAGGC (SEQ ID NO: 26);  
 GCAGGGGAAAGCAGGC (SEQ ID NO: 27); and  
 GCATACCGAAGCACGC (SEQ ID NO: 28).

27. The set of claim 24, wherein the background probes comprise short probes, wherein the probes are selected from the group consisting of

AACCATGAGAAGTATGACAA (SEQ ID NO: 29);  
 TGAGAAGTATGACAA (SEQ ID NO: 30);  
 AGTATGACAA (SEQ ID NO: 31); and  
 GACAA (SEQ ID NO: 32).

28. The method of claim 24, wherein the background probes comprise reverse polarity nucleotide analogs.



29. The method of claim 24, wherein the background probes comprise abasic phosphodiester or modified nucleotidic units.

30. A method of validating a test-background feature comprising test-background probes, said method comprising:

- (a) providing an analyte containing a target nucleotide sequence;
- (b) contacting an aliquot of said analyte containing said target nucleotide sequence with a set of features comprising oligophosphodiester probes, wherein said target nucleotide sequence is labeled with a detectable label capable of generating a measurable signal, and further wherein said features comprise
  - (i) hybridization features comprising hybridization probes that selectively hybridize to a target nucleotide sequence,
  - (ii) test-background features comprising test-background probes that do not selectively hybridize to said target nucleotide sequence, and
  - (iii) standard-background features comprising standard-background probes that do not selectively hybridize to said target nucleotide sequence;
- (c) detecting an observed signal, wherein the observed signal is an amount of signal generated from contacting the target nucleotide sequence with said features comprising oligophosphodiester probes;
- (d) detecting a test-background signal, wherein said test-background signal is an amount of signal generated from said test-background features;
- (e) detecting a standard-background signal, wherein said standard-background signal is an amount of signal generated from said standard-background features;
- (f) comparing the amount of the test-background signal with the amount of the standard-background signal.

31. The method of claim 30, wherein the wherein said target nucleotide sequence is directly labeled with said detectable label.

32. The method of claim 30, wherein the wherein said target nucleotide sequence is indirectly labeled with said detectable label.

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33. A test kit for detecting the presence and/or amount of a target nucleotide sequence in an analyte, said kit comprising a container containing an array of features comprising oligophosphodiester probes, wherein said features comprise hybridization features comprising hybridization probes that selectively hybridize to a target nucleotide sequence, and background features comprising background probes that do not selectively hybridize to said target nucleotide sequence.
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